Solvent denaturation of proteins as observed by resolution-enhanced Fourier transform infrared spectroscopy

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Summary

Fourier self-deconvolution of Fourier transform infrared (FTIR) spectra and second derivative FTIR spectroscopy were applied to study solvent-induced conformational changes in globular proteins. For β-lactoglobulin a total of three different denatured forms were identified in alkaline solution and in aqueous methanol-d_1 and isopropanol-d_1. In isopropanol-d_1 solution a new conformation was identified which appears to resemble, but is not identical with, the β-structure of native proteins. This conformation is characterized by absorption bands around 1615–1618 and 1684–1688 cm^{-1}, and is also observed for concanavalin A and chymotrypsinogen A in aqueous isopropanol-d_1 solution.

Key words: Fourier transform; deconvolution; infrared; protein conformation; solvent denaturation.

Introduction

Infrared spectroscopy is frequently used in studies of protein conformation [1–6]. The amide I band, usually observed between 1630 and 1690 cm^{-1}, is particularly useful for studies in aqueous solution [3]. In general, this band is broad and consists of unresolved components which are characteristic for specific conformations, such as the α-helix and the β-structure [4,6]. The amide I band cannot be resolved into components by increased instrument resolution because of the inherent widths of the component bands. The application of Fourier self-deconvolution [7] and of second-derivative spectroscopy [6,8], however, does result in increased resolution and permits the identification of specific conformational substructures. This study is concerned with the application of Fourier self-deconvolution and second-derivative spectroscopy to the investigation of conformational changes of β-lactoglobulin, chymotrypsinogen A and concanavalin A caused by changing the acidity of the solution and by addition of alcohol. The results indicate that these techniques constitute a useful expansion of protein conformation studies by infrared spectroscopy in general.

Materials and Methods

Bovine pancreas chymotrypsinogen A (C-4879, type II), Jack Bean concanavalin A (C-2010, type IV) and bovine β-lactoglobulin A and B (L-0130) were purchased from Sigma Chemical Co. * Deuterium oxide (99.8 atom%) and 2-propanol-d_1 (98 atom%) were supplied by Aldrich Chemical Co. Methanol-d_1 (99 atom%) was purchased from Diaprep Inc.

The samples used for spectral measurements were prepared by diluting stock solutions of proteins in ^2H_2O with appropriate deuterated solvents and were allowed to stand for at least 24 h to assure complete H,^{2}H exchange [6]. Deuterated solvents were used as in previous related work [3–6] because water absorbs very strongly at the amide I frequency [3]. About 5% (w/v) solutions were used for measurements in ^2H_2O to obtain an optimum signal-to-noise ratio [4], but concentrations below 1% (w/v) had to be used in alcohol solution to prevent aggregation [4,9,10]. Exact concentrations were measured by ultraviolet spectroscopy, using literature values for the absorptivities of β-lactoglobulin [11], chymotrypsinogen A [12], and concanavalin A [13]. Protein concentrations are given in the Figure captions.

Fourier transform infrared (FTIR) spectra were obtained with a Nicolet 7199 spectrometer equipped with a Globar source, a Ge/KBr beamsplitter, a HgCdTe detector, and a 1180 data system. 4000 interferograms at 2 cm^{-1} resolution were co-added, $1 \times$ zero-filled, apodized with the Happ-Genzel function, phase corrected, and Fourier transformed. The resulting single-beam spectra were ratioed against an empty beam background. Variable pathlength IR cells with CaF_2 windows were employed. The pathlength was set to 0.2 mm for protein concentrations below 1% (w/v), and 0.075 mm for higher concentrations. Extraordinary care was taken in purging the instrument with dry N_2, because the slightest amount of water vapor will seriously distort resolution-enhanced spectra [6]. The spectrum of the solvent was subtracted as usual from the solution spectrum. Second derivative spectra were obtained as previously described [6]. Deconvolution was carried out by the method of Kauppinen et al. [7] by computer programs adapted for the Nicolet 1180 computer. The deconvolution range was 450 cm^{-1}, from 1500 to 1950 cm^{-1}. To obtain optimum resolution without introducing artifacts [14] a program was written, which varied the resolution factor, K (as defined in Ref. 7), and the assumed half-width of the unresolved bands σ, and automatically plotted the results. ** Final

* Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

** The program is available upon request.

values of $K = 2.4$ and $\sigma = 6.5\ cm^{-1}$ were chosen for all spectra.

The upper limit of the attainable resolution improvement, K, is approximately equal to the log of the signal-to-noise (S/N) ratio of the spectra to be deconvolved [7]. Our estimated S/N ratio was > 500, sufficient for $K = 2.4$. A $2\ cm^{-1}$ resolution is necessary because some amide I components are separated by only a few wave numbers [6]. The broad deconvolution range was chosen, because we have found that better results are obtained if a portion of the flat baseline above $1700\ cm^{-1}$ is included. The same range was used for all spectra to ensure comparability.

Results and Discussion

The original FTIR spectrum of β-lactoglobulin, the self-deconvolved spectrum, and the second derivative spectrum are shown in Fig. 1A. An assignment of the resolved bands is given in Ref. 6. This protein has a high β-structure content [15], reflected by the strong $1634\ cm^{-1}$ band [3,6]. Corresponding spectra of the denatured protein at p^2H 12 are given in Fig. 1B, in acidic methanol-d_1 in Fig. 1C, and in isopropanol-d_1 in Fig. 1D. While the original protein spectra change but little from one set of conditions to another, the deconvolved spectra and the second derivative spectra show marked changes. In the deconvolved spectrum of the alkaline denatured protein (Fig. 1B, I) all fine structure disappears and a single broad band, centered at $1639\ cm^{-1}$, is observed which is assigned to a disordered structure in which the peptide groups are hydrogen bonded to the solvent [3,6]. In acidic methanol-d_1 (Fig. 1C, I) the strongest band is observed at $1647\ cm^{-1}$ and assigned to the α-helical conformation, which is known to prevail under these conditions [4,10]. Weak bands are observed at 1687 and $1618\ cm^{-1}$. In isopropanol-d_1 solution (Fig. 1D, I) relatively strong bands are observed at 1615 and $1684\ cm^{-1}$. These bands (which are close to the two weak bands observed in methanol-d_1) cannot be assigned to any known protein conformation previously studied by infrared spectroscopy [3–6]. In addition, there is ill-defined absorption between the two sharp bands which could represent some disordered structure and/or some α-helix. The β-lactoglobulin spectra given in Fig. 1A–D thus represent four different conformational states, the native form and three denatured forms. We are aware of no other spectroscopic techniques which would display these differences in such detail.

Fig. 2 displays similar data for chymotrypsinogen A. The spectrum of the native protein is displayed in Fig. 2A. A predominant β-structure is indicated by the bands at 1636 and $1674\ cm^{-1}$ [6]. Chymotrypsinogen A contains about 45% β-structure and 11% α-helix, as judged by the methods of Levitt and Greer [16]. The $1647\ cm^{-1}$ band can be assigned to the α-helix [6], as in native β-lactoglobulin (Fig. 1A), where it is observed at $1648\ cm^{-1}$. The remaining bands are probably associated with β-turns [6]. Fig. 2B shows the spectrum of chymotrypsinogen A in acidic methanol-d_1. Surprisingly, the result is quite different from the corresponding β-lactoglobulin spectrum, as shown in Fig. 1C. Instead of a dominant α-helix band, which would be observed around $1647–1652\ cm^{-1}$ [6], we observe a predominant conformation similar to the one observed for β-lactoglobulin in isopropanol-d_1 solution (see Fig. 1D). Again, a small amount of α-helix content or solvated peptide groups are

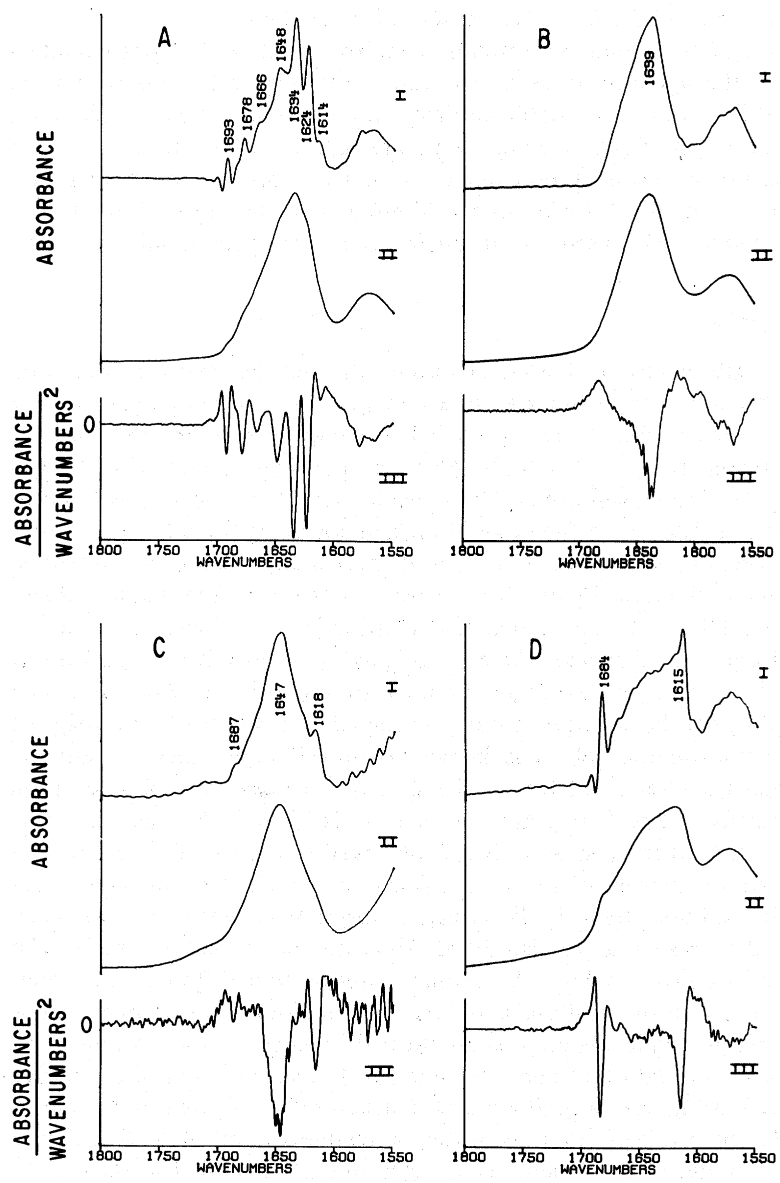

Fig. 1. Infrared spectra of β-lactoglobulin under different conditions. I, deconvolved FTIR spectrum; II, original FTIR spectrum; III, second-derivative spectrum. A, native protein in ^2H_2O, 5% (w/v) at p^2H 7; B, in ^2H_2O solution, 5% (w/v) protein at p^2H 12; C, in 60% (v/v) MeO^2H solution, 0.4% (w/v) protein at p^2H 4; D, in 40% (v/v) 2-PrO^2H solution, 0.35% (w/v) protein at p^2H 7.

indicated by ill-defined absorption centered around 1650 cm^{-1}. A similar structure is observed for this protein in isopropanol-d_1 at p^2H 7.5 as well as p^2H 2, as shown in Figs. 2C and 2D.

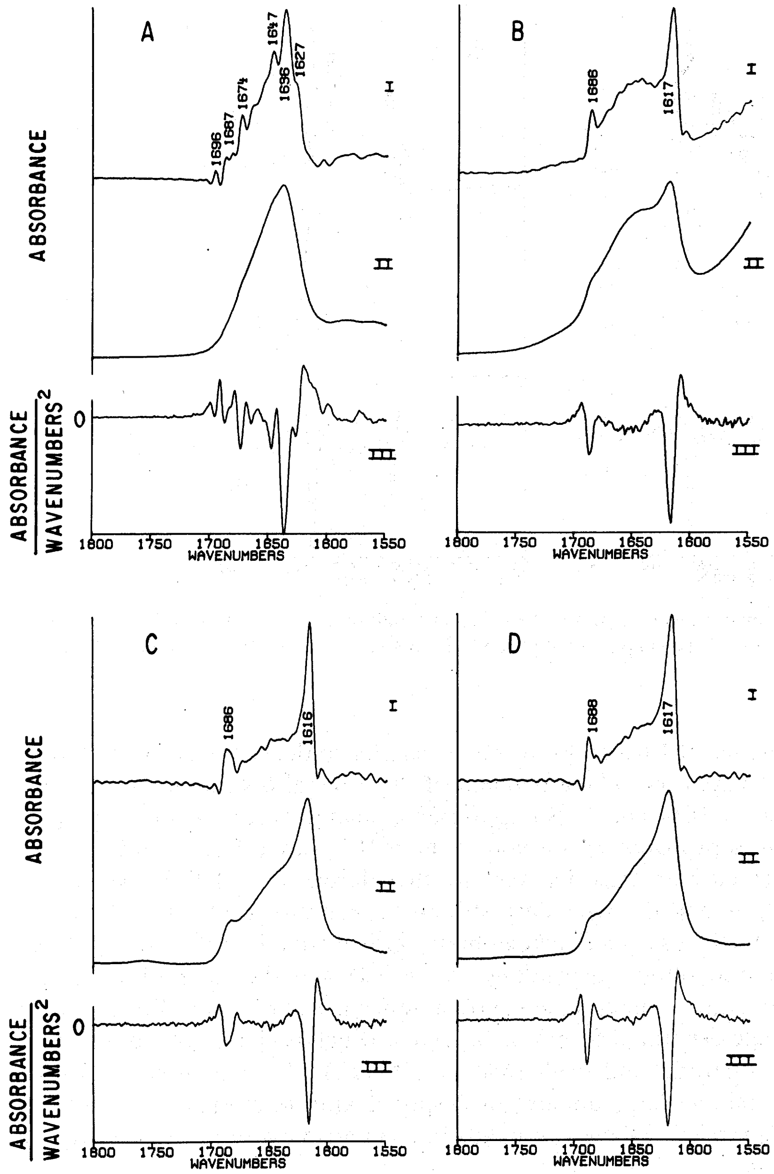

Fig. 2. Infrared spectrum of chymotrypsinogen A under different conditions. I, II and III as in Fig. 1. A, native protein in ^2H_2O, 4.7% (w/v) at p^2H 7; B, in 60% (v/v) MeO^2H solution, 0.47% (w/v) protein at p^2H 3; C, in 40% (v/v) in 2-PrO^2H solution, 0.47% (w/v) protein at p^2H 7.5; D, in 40% (v/v) in 2-PrO^2H solution, 0.4% (w/v) protein at p^2H 2.

The spectrum of native concanavalin A at p^2H 5.8 is shown in Fig. 3A. The predominant β-structure (ca. 60% by the methods of Levitt and Greer [16]) is indicated by bands at 1634 and 1670 cm^{-1}. In isopropanol-d_1 at p^2H 7 (Fig. 3B) we

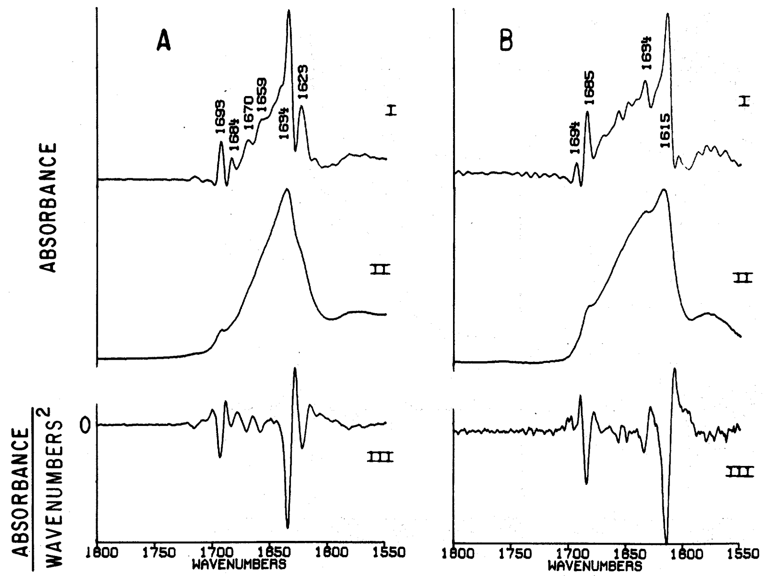

Fig. 3. Infrared spectrum of concanavalin A under different conditions. I, II and III as in Fig. 1. A, native protein in ^2H_2O solution, 5% (w/v) protein at p^2H 5.8; B, in 40% (v/v) 2-PrO^2H solution, 0.41% (w/v) protein at p^2H 7.

observe strong maxima at 1615 and 1685 cm^{-1}, similar to the two previously studied proteins. There also appears to be a remnant of the original β-structure as signified by the small band at 1634 cm^{-1}. No appreciable change occurs in the spectra of concanavalin A in isopropanol-d_1 solution as the p^2H is varied from 5 to 9.

The data presented here thus demonstrate that deconvolved FTIR spectra can clearly distinguish among the secondary structures of native proteins and various denatured forms. In the case of β-lactoglobulin three distinctly different denatured conformations were identified, depending on the p^2H and the composition of the deuterated solvent (water, aqueous methanol or aqueous isopropanol). For the other two proteins a single denatured form was observed, which is characterized by bands at 1615–1618 cm^{-1} (strong) and 1684–1688 cm^{-1} (weak). This denatured conformation probably contains a large amount of a special kind (distorted) β-strands, as judged by the frequencies of these two bands in comparison with previously studied protein spectra [1–6].

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Simplified description of the method and its applications

Fourier self-deconvolution of Fourier transform infrared (FTIR) spectra and second-derivative FTIR spectroscopy provide enhanced resolution for infrared spectra. New information concerning solvent induced conformational changes in globular proteins has been obtained. For β-lactoglobulin three different denatured conformations were identified in alkaline solution and in aqueous methanol-d_1 and aqueous isopropanol-d_1.

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